Skin Microflora

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"The dissimilarities of different regions of skin are truly profound and are necessarily reflected in the density and diversity of the organisms which inhabit them. The axilla is a tropical rain forest with its ample supplies of sweat and hair; the perineum a veritable swamp draining the cesspool of the anus. A deep, dank humid cave like the external ear canal is a sanctuary for bacterial growth. The scalp is a thick woods seeping with sebum. There are the oily tundras of the face, the comparative deserts of the trunk and the moist recesses of the intertriginous regions."[1]

The cutaneous microflora consists of a limited number of aerobic and anaerobic bacterial species. As such it stands in contrast to the highly complex flora found in other body areas such as the gastrointestinal tract, the oral cavity, and the vagina. Significant variations in both the total number of bacteria and the composition of the bacterial flora exist for different body regions. These variations reflect differences in the amount of water and nutrients available to support bacterial growth [2]. Eccrine sweat glands, which provide water, electrolytes, and minerals; apocrine sweat glands, which secrete a substance rich in protein and lipid; sebaceous glands, which produce a mixture of lipids, all contribute to the ecology of the skin. The outermost regions of the skin, the stratum corneum, is a compartment that is constantly being shed and provides a rich source of amino acids necessary for bacterial growth. Sweat glands deliver water to the surface and provide the critical moisture required for bacterial proliferation. In addition, sweat contains amino acids, and minerals such as copper, iron, magnesium, zinc, and calcium, which are important for bacterial growth and metabolism as well as toxin production by pathogenic bacteria. The presence of large amounts of water, a rich supply of proteins, lipid, and minerals, coupled with anatomy that produces semioccluded environments in some body areas and minimize evaporation of water results in an ideal ecosystem for bacterial growth. In areas of partial occlusion due to body surface-to-surface contact, such as the axilla, perineum, and toe web space, increase in CO_2 tension may also be an important factor promoting growth of bacteria [3]. The total number of aerobic bacteria found in human skin varies from 10^2 cells/cm^2 to 10^7 cells/cm^2 in wet areas such as the axilla and toe web space [2,4]. Anaerobic bacteria are found at levels of 10^4–10^6/cm^2 in areas rich in sebaceous glands, and are absent or nearly so in other body areas. The cutaneous flora consists of aerobic cocci of the micrococcaceae family, aerobic diphtheroids of the genera *Corynebacterium* and *Brevibacterium,* the anaerobic diphtheroids *Propionibacterium acnes, P granulosum,* and *P avidum,* and yeasts from the genus *Pityrosporum*. These organisms are viewed as true residents as opposed to transients in that they are found on the vast majority of people in sufficient numbers to indicate that proliferation is occurring. The skin surface is transiently visited by many organisms through environmental contamination.

AEROBIC COCCI—CLASSIFICATION OF THE MICROCOCCACEAE OF HUMAN SKIN

Acrobically growing bacteria that formed clusters attracted early attention as cutaneous inhabitants. The term staphylococcus was introduced more than 100 years ago to describe the cocci isolated from abscesses in humans, while the term micrococcus was introduced earlier for those cocci with similar morphology [5,6]. The taxonomy of these organisms has been fraught with confusion and controversy. Medical microbiologists have attempted to identify pathogenic members of this group of organisms, while taxonomists have been more concerned with fitting these organisms into a hierarchical order of classification.

Early classification of staphylococci was based on two major criteria, namely pigment formation and pathogenicity in guinea pigs [7]. From this classification came *Staphylococcus pyogenes,* which produced yellow, orange, or white pigment and was highly pathogenic in the guinea pig, and *S epidermidis,* which was white and nonpathogenic. In 1903 and 1908, *S pyogenes aureus* was shown to be capable of coagulating plasma [8,9]. In 1951 *Staphylococcus saprophyticus* was the proposed name for coagulase-negative strains, and *Micrococcus* was regarded by some as an invalid generic name [10]. In the mid-50s, there was renewed interest in the classification of staphylococci, and the demonstration that some genera could be distinguished by the ability to grow anaerobically and to produce acid from glucose formed the basis of separating staphylococci from micrococci [11]. Baird-Parker was the first to recognize heterogeneity among the coagulase-negative cocci, then referred to as *S epidermidis* [12,13]. He divided all staphylococci into six subgroups, of which group I contained *S aureus,* and referred to subgroups II through VI as *S epidermidis* biotypes 1 through 5. The subsequent application of DNA base composition analysis, usually expressed as mole-percent guanine plus cytosine, (G + C%), showed that *S aureus* and *S epidermidis* had a low (31–40%) G + C% while strains of micrococci (which are unable to grow anaerobically, and produce acid from glucose) had a high (64–74%) G + C% [14]. Others reproduced this and found a good correlation between the G + C% with the ability to grow anaerobically and produce acid from glucose, and the rationale for separating staphylococci and micrococci was fortified.

The isolation of lysostaphin provided another method for separating staphylococci from micrococci. *S aureus* and *S epidermidis*

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Abbreviations:
G + C%: moles percent guanine + cytosine
m-DAP: meso-diaminopimelic acid

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are very susceptible to lysis, while micrococci are not [15,16]. Lysostaphin susceptibility and DNA base composition are closely correlated [17,18]. Lysostaphin is an endopeptidase that splits glycyl-glycine linkages in cross-linkages of the cell wall peptidoglycan. Covalently linked to the peptidoglycan of staphylococci are teichoic acids. These water-soluble polymers make up 30–50% of the dry weight of the cell wall and contain either glycerol or ribitol joined through phosphodiester bonds. Generally, *S aureus* contains a ribitol teichoic acid with *N*-acetylglucosamine residues, and *S epidermidis* contains a glycerol teichoic acid with glucosyl residues [19]. Micrococci contain no teichoic acids, or in a few cases contain teichoic acids that are chemically and serologically distinct from those in staphylococci [20,21]. All of these findings support the current view that staphylococci and micrococci should be classified as separate genera.

Baird-Parker proposed a revised and less complex scheme for identification of the staphylococci and micrococci [22] (Table I). In 1975, Schleifer and Kloos published a series of papers redefining *S epidermidis* and *S saprophyticus* [23–25]. Of the four biotypes of *S epidermidis* and the four of *S saprophyticus,* only biotype 1 was named *S epidermidis* and biotype 3 named *S saprophyticus.* Many new species were recognized on the basis of extensive morphologic, physiologic, and biochemical characters. *S epidermidis* group was classified as *S epidermidis, S capitis, S warneri,* and *S hominis,* while the *S saprophyticus* group was classified as *S saprophyticus, S xylosus,* and *S cohnii.*

Using the new classification, *S epidermidis* and *S aureus* are the most frequently isolated organisms from the anterior nares, while *S epidermidis* and *S hominis* are the most frequently isolated staphylococci from the axillae, head, legs, and arms [26].

Aerobic Coryneforms The aerobic coryneforms are a major component of the normal skin flora; however, these bacteria present a number of unresolved problems in taxonomy and classification. Aerobic coryneforms, known in the medical literature as diphtheroids, show little if any resemblance to the pathogen *Corynebacterium diphtheriae* except for morphology [27]. Members of the genus *Corynebacterium* are gram-positive, pleomorphic rods, characterized by the presence of meso-diaminopimelic acid (*m*-DAP) as the diaminoacid of the peptidoglycan, and by the presence of arabinose, galactose, and often mannose in the cell wall polysaccharide. Specifically the presence of corynemycolic acids (C_{20}–C_{38}) in the cell walls of *Corynebacterium subspecies* provide a taxonomic tool for separation of these organisms from members of the genus *Brevibacterium.* The brevibacteria typically posess a cell wall with *m*-DAP with galactose (Table II). It has been reported that brevibacteria from human skin and from dairy products are similar to the type strain *B linens* [29]. Much weight was assigned to the ability to produce methane-thiol (CH_3SH) from L-methionine by both skin and dairy isolates [30]. However, Collins et al [31] demonstrated that skin and dairy isolates form two distinct DNA homology groups, both of which show little relatedness to the type species *B linens.* They propose the classification of skin isolates as *B epidermidis,* species novum, distinct from the dairy isolates *B casei,* species novum.

Table I. Taxonomy of Aerobic Cocci

Characteristic	Staphylococcus	Micrococcus
DNA G+C%	30–40	66–75
Cell Wall:		
Glycine crosslink	+	–
Teichoic acids	+	–
Anaerobic:		
Glucose fermentation	+	–
Lysostaphin susceptibility	+	–
Erythromycin-sensitive (0.4 mg/ml)	–	+

Source: Baird-Parker [22].

Table II. Cell Wall Composition in the Taxonomy of Cutaneous Aerobic Diphtheroids

Component	Brevibacterium	Corynebacterium
m-DAP	+	+
Arabinose	–	+
Galactose	+	–
Mycolic acids	–	+
Fatty acids (major type)	Branched	Straight
Teichoic acids	+	–

Source: Bousfield et al [28].

Aerobic diphtheroids have also been subdivided into lipophilic and nonlipophilic strains [32]. Lipophilic diphtheroids are most plentiful in moist body regions such as the axillae, perineum, toe web space, and are abundant in areas of less body occlusion but rich in eccrine sweat glands, such as the scalp and nares. The density of these organisms varies from thousands per square centimeter on the extremities to millions per square centimeter in perineum and toe web space [2–4]. These organisms have a strict nutritional requirement for lipid and cannot proliferate in its absence [33]. Analysis of cell wall mycolic and fatty acids indicate that these lipophilic diphtheroids are similar to well defined species of *Corynebacterium* [33]. However, it has been shown that lipophilic diphtheroids are distinct from the classical group of coryneforms, as they exhibit a strict nutrional requirement for lipid not shared by *C minutissimum* or *C xerosis* and differ from strains of *C bovis* and *C xerosis* in their cell wall fatty acid and mycolic acid composition. The species of *Corynebacterium lipophilicus* has been proposed for the aerobic lipophilic diphtheroid found on human skin [33].

Anaerobic Coryneforms The anaerobic gram-positive diphtheroids that live on human skin have, until recent years, been the subject of great confusion. In 1966, Prevot and Fredette listed a total of 12 anaerobic coryneforms [34]. In 1972, Johnson and Cummins used cell wall analysis, DNA base compositions, and DNA homology determinations to study the group of anaerobic coryneforms [35]. They found that all strains fell into the genus *Propionibacterium* and comprised three different species: *P acnes, P avidum,* and *P granulosum. P acnes* and *P avidum* were divided into two subgroups on the basis of cell wall sugar content. These subspecies were later shown to be detectable with appropriate antisera, and are referred to at present as serotypes I and II in each species [36]. Later work demonstrated that the three species proposed by Johnson and Cummins could be differentiated on the basis of conventional biochemical tests [37,38]. They also found that *P acnes* serotype I strains could be presumptively identified on the basis of sorbitol fermentation. Since a proportion of group I strains fail to ferment sorbitol, a negative test does not indicate a group II strain. The differences in serotype and species are also reflected in the electrophoretic mobility of malate dehydrogenase and, in *P acnes,* in bacteriophage susceptibility [39,40] (Table III). Of interest is that most strains labelled *Corynebacterium parvum,* which have been used as immunostimulants in animals and humans are *P acnes* [41].

Pityrosporum The only yeast genus found in significant numbers on humans is *Pityrosporum.* The three morphologic types found on the skin, oval, round, and hyphal, gave rise to the delineation of three separate species: *Pit ovale, Pit orbiculare,* and *Malassezia furfur.* Recent work has demonstrated that these three forms are actually morphologic variants of the same species [42]. They are antigenically similar [43,44], have similar nutritional requirements, and are interconvertible [45,46].

Anaerobic Cocci The presence of anaerobic staphylococcus has also been demonstrated on the skin [47]. *Peptococcus sacchar-*

Table III. Biochemical Reactivities and Cell Wall Composition of Propionibacteria

Species	Biochemical Reactions							Cell Wall Composition				
	Ind	Nit	Cat	Gel	Sor	B-haem	LMA	G+C%	Dap	Gal	Glu	Man
P acnes												
I	+	+	+	+	V	−	+	57–60	LL	+	+	+
II	+	+	+	+	−	−	+		LL or m	−	+	+
P avidum												
I	−	−	+	+	−	+	++	62–63	LL	+	+	+
II	−	−	+	+	−	+	++		LL or m	−	+	+
P granulosum	−	−	+	−	−	−	−	62–64	LL	+	V	+

Ind = indole, Nit = nitrate, Cat = catalase, Gel = gelatinase, Sor = sorbitol fermentation, B-Haem = b-haemolysis, LMA = litmus milk agar test, Dap = diaminopimelic acid, Gal = galactose, Glu = glucose, Man = mannose, LL = LL-diaminopimelic acid, m = meso-diaminopimelic acid, V = variable.
Source: Johnson et al [35], Pulverer et al [37], Fergusin et al [38].

olyticus can be recovered in significant numbers on approximately 20% of subjects. The *Pepto saccharolyticus* strains are capable of scanty aerobic growth, nonhemolytic, usually catalase positive, and ferment glucose, fructose, and glycerol, but not maltose. The strains tested were coagulase negative and lysostaphin positive. The exact ecologic niche of this organism remains to be determined.

POPULATION DYNAMICS AND ECOLOGY

Different regions of the skin surface have been shown to have reproducible, stable, and distinct bacterial populations. Both the density of colonization and the constituent species of a site can be interpreted to identify unique cutaneous environments. Examples of such environments include the sebaceous regions, predominantly inhabited by *P acnes* and *Pit ovale,* and the wetlands of the axillae and groin, which harbor large numbers of lipophilic diphtheroids and micrococci, and lower numbers of transient gram-negative bacteria.

Three major determinants of cutaneous habitats have been identified: ability to maintain a reduced environment, availability of moisture, and the presence of sebaceous lipid. The effects of these factors are clearly reflected in the microbial populations of dry, oily, and wet regions (Tables IV, V, and VI, respectively).

Oily regions, such as the head, upper back, and trunk are sebaceous gland–rich areas. Sebaceous follicles can supply two important needs of the resident bacteria, a lipid growth substrate and a protected niche that can maintain an anaerobic environment. The dominant bacteria in these regions are the *Propionibacteria: P acnes* and, to a lesser extent, *P granulosum* [49]. These microaerophilic organisms live in the depths of the sebaceous follicle, where a reduced environment is maintained presumably through bacterial metabolism. The primary growth substrate is almost certainly sebaceous triglycerides. *P acnes* population densities are proportional to the amount of secreted sebum, and the amount of free fatty acids in skin surface lipid, and are inversely proportional to the level of free glycerol in sebum [50,51].

Although closely related to *P acnes, P avidum* has a distinct preference for moist areas. It may be isolated in greatest numbers from the anterior nares, perineum, and axilla [49,52]. *P avidum* is lipolytic, but also has much greater proteolytic activity than *P acnes* and *P granulosum* and may thus readily metabolize nonlipid cutaneous substrates, possibly accounting for its presence in the axilla, anterior nares, and perineum. It also parallels the cutaneous distribution of gram-negative enterics, which may suggest an intestinal reservoir for the organism.

The other major inhabitant of sebaceous regions is the yeast *Pit ovale. Pit ovale* is the most numerous on the scalp [53] and, like *P acnes,* it is believed to derive substrates through lipolytic cleavage of sebaceous triglycerides.

In nonsebaceous regions, the major environmental factor appears to be the availability of water. Areas that are rich in sweat glands and restricted in air flow, such as the intertriginous regions, are oases on the otherwise arid skin surface. They support large populations of the "hydrophilic" skin bacteria, including aerobic diphtheroids and micrococci, some gram negatives, and *P avidum.* The importance of water can be experimentally demonstrated by artificially hydrating a naturally dry region, such as the forearm. Within 24 h the normal population expands from 10^3 to 10^6 organisms/cm^2 and exhibits a large increase in the proportion of staphylococci and a concomitant decrease in the proportion of the normally numerous micrococci. Extended hydration (7 days) results in a tripling of the 24-h bacterial density, a predominance of lipophilic diphtheroids, and significant levels of gram negatives and "large colony" diphtheroids [54]. This population now closely resembles that found on the foot, an area that becomes hydrated when occlusive shoes are worn.

The relative potency of sebum and water availability as ecologic determinants has been addressed in a study of the effect of occlusion on scalp flora. As previously discussed, when nonsebaceous regions are occluded, there is a great increase in bacterial numbers and a change in the character of the resident organisms. This does not occur after occlusion-hydration of the scalp [53], suggesting that a flora adjusted to growth in sebum, or products of the sebaceous flora prevent colonization by hydrophilic species.

Table IV. Microbial Flora of Dry Regions

	Upper extremeties	Lower extremeties
Sample number	49	38
Density[a]	1.7×10^3	4.4×10^3
Composition (%)		
Cocci	93.1	87.8
Lipophilic diphtheroids	3.9	5.0
Large-colony diphtheroids	0	7.1
Propionibacteria	3.0	0.01

[a]Geometric mean/cm^2.

Table V. Microbial Flora of the Oily Regions

	Scalp		Normal forehead
	Nondandruff	Dandruff	
Sample	112	126	76
Density[a]	1.0	1.2	4.4
Composition (%)			
Cocci	23.1	18.8	7.2
Lipophilic diphtheroids	2.3	0.9	0.02
Large-colony diphtheroids	0	0.1	0
Gram-negative rods	0.002	0.001	0
Propionibacteria	27.5	6.1	83.5
Pityrospora	46.7	73.9	9.2

[a]Geometric mean/cm^2 ($\times 10^6$).
Source: McGinley et al [48,49].

Table VI. Microbial Flora of the Wet Regions of the Skin

	Axilla			Foot Interspace		
	Nonapocrine Odor	Apocrine Odor	Normal Perineum	Normal	Dermatophytosis Simplex	Dermatophytosis Complex
n	16	27	30	48	39	101
Density[a]	4.8×10^3	1.3×10^6	4.3×10^7	1.4×10^7	2.9×10^7	1.1×10^8
Cocci[b]	86.6	25.7	14.2	22.7	12.9	10.0
Lipophilic diphtheroids	10.9	54.7	58.0	75.1	78.1	31.4
Large colony diphtheroids	0.3	16.9	26.4	2.1	8.8	56.5
Gram-negative rods	1.1	0.3	7.7	0.05	0.06	2.04
Propionibacteria	1.1	2.4	0	0	0	0
Candida subspecies	0	0	0.06	0.02	0.02	0.04
Dermatophytes[c]	0	0	0	0	84.6	46.0

Source: Leyden et al [69], Leyden et al [83].
[a]Geometric mean colony-forming units per square centimeter.
[b]Percent composition of sample.
[c]Prevalence.

A recently recognized phenomenon is the change in bacterial populations with age. A significant production of sebum begins at puberty, peaks in early adulthood, and declines in old age. A similar pattern of age-related carriage in *P acnes* populations has been reported on the face [55].

The normal flora itself also is a major force in determining the nature of the microenvironment. Fatty acids cleaved from sebaceous triglycerides has been shown to be inhibitory to strains of *Streptococcus pyogenes,* and presumably afford a measure of protection to the host [56].

Another way in which skin bacteria may influence the composition of the skin flora is through the production of antibiotics or bacteriocins. Selwyn and Ellis detected bacteria that produce inhibitory compounds on 20% of normal adults [57]. Later work has revealed a strain of *S epidermidis* that produces a factor that kills strains of *Micrococcus, C diphtheriae,* and *Streptococcus pyogenes* [58].

Although the in vivo significance of these phenomena has not been established, there are suggestive findings. The demonstration that eradication of the normal flora greatly enhanced the survival of a *S aureus* inoculation and the subsequent development of infection suggests that the resident flora acts as a line of defense against infection [59]. This phenomenon may be operative in the staphylococcal colonization of neonates. The newborn's skin is virtually sterile at birth and a normal flora is not established for several days. It is during this period that the highest incidences of staphylococcal infections has been reported [60].

Bibel et al [61] inoculated a bacitracin-producing strain of *Bacillus licheniformis* to human skin. A shift to bacitracin resistance in the normal flora was demonstrated, suggesting a role for antibiotic production in the maintenance of the normal flora.

The resident flora does not appear to provide protection against infection by *Candida albicans, Strep pyogenes,* or *Pseudomonas aeruginosa*. In the case of *Candida albicans,* moisture appears to be the critical factor for survival [62,63]. Invasion of the skin occurs as the filamentous phase develops [64]. Subsequently, the alternative pathway of complement is activated, polymorphonuclear leukocytes are recruited, with the result being inflammation and pustule formation [65]. The stratum corneum provides the first line of defense, which can be surmounted by proliferation of *Candida subspecies* and filament development. In the case of *Strep pyogenes,* survival and growth on intact human skin is brief, and infection does not occur in the absence of a break in the stratum corneum [66]. *Pseudomonas aeruginosa* will survive on intact human skin provided sufficient moisture is present, but infection does not occur in the absence of damage to the stratum corneum, again demonstrating the significance of the stratum corneum in the prevention of cutaneous infection [67].

Another area in which the resident microflora has been shown to be of importance is in the production of body odor. Axillary odor has received the most intensive study where the interaction of resident surface bacteria and sterile odorless apocrine sweat produce the characteristic malodor of the human axilla [68]. The bacteria that produce this odor are aerobic diphtheroids [69].

RESIDENT FLORA AS PATHOGENS

Micrococcaceae *S aureus* is the most prominent member of the *Micrococcaceae* with a recognized potential for pathogenicity. *S aureus* has been reported as the etiologic agent in suppurative conditions such as impetigo, boils, and wound infections [4]. Distinguishing between colonization and infection is of great importance, as nasal carriage rates vary from less than 10% to more than 40% in the normal population outside hospitals [70,71]. Colony counts of *S aureus* less than 10^6 organisms/cm^2 are considered to represent colonization without infection. The degree of colonization correlates well with the severity of exudation of the dermatitis. *S aureus* is nearly always present in atopic dermatitis, numular eczema, and neurodermatitis. Only some 20% of patients with seborrheic dermatitis are colonized by *S aureus,* and in psoriasis the organism is present in low numbers in some 50% of cases. Exfoliation of squames that are carrying a high *S aureus* population may pose a serious threat to hospitalized patients [71].

Excellent reviews have been published on scalded skin syndrome [72–74]; however, caution should be exercised in attributing all of these to staphylococcal etiology. Intravenous, intraperitoneal, or subcutaneous doses of toxin in adult hairless mice has been shown to induce splitting of the skin. The toxin has been demonstrated in the blister fluid of children with bullous impetigo [75].

There is no evidence to clearly define any pathogenic role for other members of the Micrococcaceae in cutaneous infections. There are, however, three situations in which a pathogenic role can be clearly defined for coagulase-negative cocci: bloodstream and urinary tract infections, and infection of surgical prostheses [76–78]. The ability of some of the coagulase-negative cocci to produce a mucoid substance that enables them to adhere to glass, metal surfaces, and catheters may be an important factor determining whether these normally nonpathogenic organisms can cause infection.

Aerobic Diphtheroids Aerobic diphtheroid bacteria have been isolated from a variety of skin infections such as erythrasma, pitted keratolysis, and trichomycosis axillaris.

Erythrasma is a superficial infection with scaling, and is commonly seen in toeweb spaces and other intertriginous areas. Coral red fluorescence is seen under Wood's light, and is due to the presence of porphyrins produced by skin bacteria. This infection is viewed as being caused by a specific organism, *C minutissimum*

[79], although several species of fluoresence-producing diphtheroids and nonfluorescent diphtheroids may be involved [80].

Aerobic diphtheroids have also been implicated as the causative agent of pitted keratolysis; a superficial infection of the stratum corneum usually limited to the soles and only occasionally seen on the palms. A characteristic erosion of the stratum corneum leads to the production of typical pits. A diphtheroid organism has reportedly been isolated from this condition and has been used to reproduce the pitting in healthy volunteers [81]. Identification of the organism as a member of the genus *Corynebacterium* was based on morphologic criteria and a G + C% of 59.4%. However, it is important to recognize that DNA base ratios for the coryneform group of bacteria are considered to be of limited taxonomic value in the absence of other confirming taxonomic characteristics, as a wide range of G + C% values occur in this group of bacteria [82]. Therefore, it can be concluded that, at the present time, there is not enough evidence to implicate any one particular organism as the etiologic agent of pitted keratolysis.

Aerobic diphtheroids also appear to play an important, although secondary role in interdigital athlete's foot [83]. Prolonged hydration favors a great increase in large-colony diphtheroids, and products of these organisms diffuse through the weakened barrier of the stratum corneum and cause the symptomatic disease.

Overgrowth of a variety of diphtheroids in trichomycosis axillaris characteristically produces waxy growths of varying color on hair shafts in the axilla and less commonly on pubic and beard hair. These organisms produce keratinolytic enzymes and invade the hair shaft cuticle [84,85].

It is not clear whether these aerobic diphtheroids are species of organisms that are normally found on human skin and are acting in a pathogenic fashion, or whether they are nonresident organisms that are inherently pathogenic and have colonized the skin surface. Until relatively recently, the isolation of nondiphtheria coryneforms from hospitalized patients was usually viewed as nonsignificant contamination. Subsequently, however, several reports have documented the emergence of multiple antibiotic resistant coryneform bacteria (group JK) as a cause of nocosomial infection, including at least one epidemic, and have involved patients clearly at risk for infection due to immunosuppressive therapy or neutropenia [86,87]. Interestingly, the pathogenic group JK coryneforms that are resistant to multiple antibiotics, and the antibiotic-sensitive cutaneous lipophilic diphtheroids share a strict nutritional requirement for lipid and a similar cell wall fatty acid, mycolic acid, and sugar composition [88]. Furthermore, a significant correlation exists between the proportion of the antibiotic-resistant group JK and antibiotic-susceptible lipophilic diphtheroids. As the relative density of group JK coryneforms increases, the density of the lipophilic diphtheroids decreases [89] (Table VII). Therefore it appears that the group JK coryneform bacteria are lipophilic diphtheroids that have acquired multiple resistance to antibiotics.

Table VII. Prevalence of Antibiotic-sensitive Lipophilic Diphtheroids and Antibiotic-resistant Group JK Coryneforms in Hospitalized and Nonhospitalized Populations

	Site (percent prevalence)			
	Nose	Axilla	Perineum	Toeweb
Hospitalized patients (n = 43)				
JK	65.1	48.8	69.8	67.4
LD	34.9	55.8	65.1	72.1
Healthy adults (n = 80)				
JK	0	0	15.0	6.3
LD	90.0	70.0	98.7	93.8

Source: Larson et al [89].

Anaerobic diphtheroids There is substantial evidence that the anaerobic diphtheroid *P acnes* plays an important role in the pathogenesis of inflammation in acne vulgaris. The evidence includes the following: (1) Patients with teenage acne have significantly higher densities of *P acnes* recovered from their skin than age-matched controls [90]. (2) Successful suppression of *P acnes* by systemic or topical antibiotics is accompanied by clinical improvement [91,92]. (3) *P acnes* elaborates diffusible neutrophil chemotactic factors that are predominantly low in molecular mass [93,94]. (4) *P acnes* activates both the classical and the alternative pathways of complement to produce C_5-derived neutrophil chemotactic factors [95–97]. Bound C_3 has been reported in the tissue surrounding inflammatory acne lesions [98]. The alternative pathway activator has been identified as a cell wall carbohydrate [95]. (5) Ingestion of *P acnes* by neutrophils results in the extracellular release of lysozomal hydrolases [96]. Similar release of lysosomal enzymes has been implicated in the tissue destruction in periodonatal disease and rheumatoid arthritis. (6) Intradermal injection of *P acnes* is more inflammatory in acne patients than in controls [97]. (7) *P acnes* antibody titers parallel the severity of inflammation in acne [97] and, in vitro, modulate the magnitude of complement activation [95,99]. In addition to its role in acne, *P acnes* is also a frequent opportunistic pathogen, and is isolated from wound infection, osteomyelitis, and endocarditis [100–103]. Several reports of meningitis exist, and botryomycosis due to *P acnes* has recently been reported. Perhaps the most significant infectious role of *P acnes* is as a postneurosurgical pathogen [104–107]. The proximity of the scalp reservoir of *P acnes* to the site of incision doubtless is the source of contamination. The inability to sterilize the depths of sebaceous follicles in preoperational preparations also prevents the clearance of *P acnes* from the site of incision.

Pityrosporum The current weight of evidence indicates that the increase in *Pit ovale* on the scalps of those with dandruff is secondary to the process and not causative [108]. *Pit ovale* has an invasive phase consisting of short hyphae that invade the skin superficially causing tinea versicolor [109]. The cutaneous ecologic factors that promote this dimorphism are unknown. The recent finding that cholesterol and cholesterol esters are necessary for in vitro development of the filamentous phase may be of significance for in vivo events [46]. The pigmentary disturbances frequently seen in tinea versicolor (usually hypopigmentation or failure to tan) have been shown to be due to tyrosinase inhibition and damage to melanocytes by C_9 and C_{12} dicarboxylic acids formed by the oxidation of the double-bond linkages of unsaturated fatty acids in skin lipids by a *Pityrosporum* enzyme system [110].

Our knowledge of the cutaneous flora, the factors that determine which organisms will colonize skin, and how pathogenic bacteria penetrate host defenses has progressed from Professor Kligman's statement "We have come to realize since our last review in 1954, how uncertain much of our knowledge is of the cutaneous flora."

REFERENCES

1. Kligman AM: The bacteriology of normal skin, in Skin Bacteria and Their Role in Infection. Edited by HI Maibach, G Hildrich-Smith. New York, McGraw Hill, 1965, pp 13–31
2. Leyden JJ, McGinley KJ, Webster GF: Cutaneous microflora, in Biochemistry and Physiology of the Skin. Edited by LA Goldsmith. New York, Oxford University Press, 1983, pp 1153–1165
3. Aly R, Shirley C, Curnico B, Maibach HI: Effect of prolonged occlusion in the microbial flora, pH, carbon dioxide and transepidermal water loss in human skin. J Invest Dermatol 71:378–381, 1978
4. Noble WC: Microbiology of Human Skin, London, Lloyd-Luke, 1981, pp 339–357
5. Ogston A: Micrococcus poisoning. J Anat Physiol 17:24–58, 1883

6. Cohn F: Untersuchungen uber Bacterien II. Beitr Biol Phalanz 1: 127–224, 1875
7. Andrewes FW, Gordon MH: Report on the biological characters of the staphylococci pathogenic for man. Rep Med Off Loc Govt Bd App B 7:543–560, 1905
8. Loeb L: The influence of certain bacteria on the coagulation of the blood. J Med Res 10:407–419, 1903
9. Much H: Uber eine Vorstufe des Fibrinfermentes in Kulturen von *Staphylokokkus aureus*. Biochem J 14:143–155, 1908
10. Shaw C, Stitt JM, Cowan ST: Staphylococci and their classification. J Gen Microbiol 5:1010–1023, 1951
11. Evans JB, Bradford WL, Niven CF Jr: Comments concerning the taxonomy of the genera *Micrococcus* and *Staphylococcus*. Int Bull Bacteriol Nomencl Taxon 5:61–66, 1955
12. Baird-Parker AC: A classification of micrococci and staphylococci based on physiological and biochemical tests. J Gen Microbiol 30:409–427, 1963
13. Baird-Parker AC: Staphylococci and their classification. Ann NY Acad Sci 128:4–25, 1965
14. Belozersky AN, Spirin AS: Chemistry of the nucleic acids of microorganisms, in The Nucleic Acids. Edited by E Chargaff, JN Davidson. London, Academic Press, 1960, pp 147–185
15. Schindler CA, Schuhardt VT: Lysostaphin: a new bacteriolytic agent for the staphylococcus. Proc Natl Acad Sci USA 51:414–421, 1964
16. Schuhardt VT: Discussion of the paper; staphylococci and their classification. Ann NY Acad Sci 128:19–25, 1965
17. Klesius PH, Schuhardt VT: Use of lysostaphin in the isolation of highly polymerized deoxyribonucleic acid and in the taxonomy of aerobic micrococcaceae. J Bacteriol 95:739–743, 1968
18. Lachica RVG, Hoeprich PD, Genigeorgis C: Nuclease production and lysostaphin susceptibility of *Staphylococcus aureus* and other catalase positive cocci. Appl Microbiol 21:823–826, 1971
19. Davison AL, Baddiley J: The distribution of teichoic acids in staphylococci. J Gen Microbiol 3:271–276, 1963
20. Badilley J, Brock JH, Davison AL, Partridge MD: The wall composition of micrococci. J Gen Microbiol 54:393–396, 1968
21. Oeding P, Mylkestad B, Davison AL: Serologic investigation on teichoic acids from the walls of *Staphylococcus epidermidis* and *Micrococcus*. Acta Pathol Microbiol Scand 69:458–464, 1967
22. Baird-Parker AC: The basis for the present classification of staphylococci and micrococci. Ann NY Acad Sci 236:7–14, 1974
23. Kloos WE, Schleifer KH: Isolation and characterization of staphylococci from human skin. II. Description of four new species: *Staphylococcus warneri, Staphylococcus capitis, Staphylococcus hominis,* and *Staphylococcus simulans*. Int J Syst Bacteriol 25:62–79, 1975
24. Kloos WE, Schleifer KH: Simplified scheme for routine identification of human *Staphylococcus* species. J Clin Microbiol 1:82–88, 1975
25. Schleifer KH, Kloos WE: Isolation and characterization of staphylococci from human skin. I. Amended description of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* and descriptions of three new species: *Staphylococcus cohnii, Staphylococcus haemolyticus,* and *Staphylococcus xylosus*. Int J Syst Bacteriol 25: 50–61, 1975
26. Kloos WE, Musselwhite MS: Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin. Appl Microbiol 30:381–395, 1975
27. Pitcher GP, Jackman PJH: The current status of aerobic cutaneous coryneform bacteria, in Skin Microbiology, Relevance to Clinical Infection. Edited by HI Maibach, R Aly. New York, Springer-Verlag, 1985, pp 19–28
28. Bousfied IJ, Calley AG: Coryneform Bacteria. New York, Academic Press, 1978, pp 265–287
29. Sharpe ME, Law BA, Phillips BA, Pitcher DG: Methanethiol production by coryneform bacteria: strains from human and skin sources and *Brevibacterium linens*. J Gen Microbiol 101:345–349, 1977
30. Sharpe ME, Law BA, Phillips BA, Pitcher DG: Coryneform bacteria producing methanethiol, in Special Publications for the Society for General Microbiology, I. Coryneform Bacteria. Edited by IJ Bousfield, AG Callely. London, Academic Press, 1978, pp 289–300
31. Collins MD, Farrow JA, Goodfellow M, Minnikin DE: *Brevibacterium casei* sp. nov. and *Brevibacterium epidermidis* sp. nov. System Appl Microbiol 4:388–395, 1983
32. Evans NM: The classification of aerobic diphtheroids from human skin. Br J Dermatol 80:81–91, 1968
33. McGinley KJ, Labows JN, Zehman JM, Nordstrom KM, Webster GF, Leyden JJ: Analysis of cellular components, biochemical reactions and habitat of human cutaneous lipophilic diphtheroids. J Invest Dermatol 85:374–377, 1985
34. Prevot A, Fredette V: Manual for the Classification and Determination of Anacrobic Bacteria. Philadelphia, Lee and Febiger, 1966, pp 374–382
35. Johnson JL, Cummins CS: Cell wall composition and deoxyribonucleic acid similarities among the anaerobic coryneforms, classical corynebacteria, and strains of *Arachnia propionica*. J Bacteriol 109:1047–1066, 1972
36. Cummins CS: Identification of *Propionibacterium acnes* and related organisms by precipitin tests with trichloracetic acid extracts. J Clin Microbiol 2:104–110, 1975
37. Pulverer G, Ko HL: Fermentative and serological studies on *Propionibacterium acnes*. Appl Microbiol 25:222–225, 1973
38. Fergusson DA, Cummins CS: Nutritional requirements of anaerobic coryneforms. J Bacteriol 135:858–867, 1978
39. Gross CS, Ferguson DA, Cummins CS: Electrophoretic protein patterns and enzyme mobilities in anaerobic coryneforms. Appl Environm Microbiol 35:1102–1109, 1978
40. Webster GF, Cummins CS: The use of bacteriophage typing to differentiate *Propionibacterium acnes* type I and II. J Clin Microbiol 7:84–90, 1978
41. Cummins CS, Johnson JL: *Corynebacterium parvum:* a synonym for *Propionibacterium acnes*. J Gen Microbiol 80:433–422, 1974
42. Salkin IF, Gordon MA: Polymorphism of *Malessezia furfur*. Can J Microbiol 23:471–475, 1977
43. Webster GF, McGinley KJ: Serologic analysis of the extractable carbohydrate antigens of *Pityrosporum ovale*. Microbios 28:41–45, 1980
44. Tanaka M, Imamura S: Immunological studies on the *Pityrosporum* genus and *Malessezia furfur*. J Invest Dermatol 73:321–324, 1979
45. Dorn M, Roehnert K: Dimorphism of *Pityrosporum orbiculare* in a defined culture medium. J Invest Dermatol 69:244–248, 1977
46. Nazzaro-Porro M, Passi S, Caprilli F, Mercantini R: Induction of hyphae in cultures of *Pityrosporum* by cholesterol and cholesterol esters. J Invest Dermatol 69:531–534, 1977
47. Leyden JJ, Kligman AM: The role of micro-organisms in diaper dermatitis. Arch Dermatol 119:56–59, 1978
48. McGinley KJ, Leyden JJ, Marples RR, Kligman AM: Quantitative microbiology of the scalp in non-dandruff, dandruff and seborrheic dermatitis. J Invest Dermatol 64:40–46, 1975
49. McGinley KJ, Webster GF, Leyden JJ: Regional variations of cutaneous propionibacteria. Appl Env Microbiol 35:62–65, 1978
50. McGinley KJ, Ruggieri MR, Webster GF, Leyden JJ: Regional variations in density of cutaneous propionibacteria: correlation of *P. acnes* populations with sebaceous secretion. J Clin Microbiol 12: 672–675, 1980
51. Rebello T, McLeod Hawk JL: Skin surface glycerol levels in acne vulgaris. J Invest Dermatol 70:352–354, 1978
52. Nordstrom NKM: Colonization of the axilla by *Propionibacterium avidum* in relation to age. Appl Environm Microbiol 47:1360–1362
53. Kligman AM, Leyden JJ, McGinley KJ: Bacteriology. J Invest Dermatol 67:160–168, 1976
54. Marples RR: The effect of hydration of the bacterial flora of the skin, in Skin Bacteria and Their Role in Infection. Edited by HI Maibach, G Hildick-Smith. New York, McGraw-Hill, 1967, pp 33–41

55. Leyden JJ, McGinley KJ, Mills OH, Kligman AM: Age related changes in the resident bacterial flora of the human face. J Invest Dermatol 65:379–381, 1975
56. Aly R, Maibach HI, Strauss WG, Shinefeld HR: Survival of microorganisms on human skin. J Invest Dermatol 58:205–210, 1972
57. Selwyn S, Ellis H: Skin bacteria and skin disinfection reconsidered. Br Med J 1:136–140, 1972
58. Milyani RM, Selwyn S: Quantitative studies on competitive activities of skin bacteria growing on solid media. J Med Microbiol 11:379–386, 1977
59. Singh G, Marples RR, Kligman AM: Staphylococcus infections in humans. J Invest Dermatol 57:149–162, 1971
60. Hurst V: Transmission of hospital staphylococci among newborn infants. Pediatrics 25:204–214, 1959
61. Bibel DJ, Smiljanic RJ, Lovell DJ: Interactions of *Bacillus licheniformis* ATCC 10716 and normal flora of human skin. Appl Env Microbiol 35:1136–1144, 1978
62. Rebora A, Marples RR, Kligman AM: Experimental infection with *Candida albicans*. Arch Dermatol 108:69–73, 1973
63. Maibach H, Kligman AM: The biology of experimental human cutaneous moniliasis (*Candida albicans*). Arch Dermatol 85:233–257, 1962
64. Ray TL, Wuepper KD: Experimental cutaneous candidiasis in rodents. II. Role of the stratum corneum barrier and serum complement as a mediator of a protective inflammatory response. Arch Dermatol 114:539–543, 1978
65. Ray TL, Wuepper KD: Activation of the alternative (properdin) pathway of complement by *Candida albicans* and related species. J Invest Dermatol 67:700–703, 1976
66. Leyden JJ, Stewart R, Kligman MA: Experimental infections with group A streptococci in humans. J Invest Dermatol 75:196–210, 1978
67. Leyden JJ, Stewart R, Kligman AM: Experimental inoculation of *Pseudomonas aeruginosa* and *Pseudomonas cepaciae* on human skin. J Soc Cos Chem 31:19–29, 1980
68. Labows JL, Preti G, Hoelzle E, Leyden JJ, Kligman AM: Steroid analysis of human apocrine secretion. Steroids 34:249–258, 1979
69. Leyden JJ, McGinley KJ, Hoelzle E, Labows JN, Kligman AM: The microbiology of the human axilla and its relationship to axillary odor. J Invest Dermatol 77:413–416, 1981
70. Williams REO: Healthy carriage of *Staphylococcus aureus:* its prevalence and importance. Bact Reviews 27:56–71, 1963
71. Leyden JJ, Marples R, Kligman AM: *Staphylococcus aureus* in the lesions of atopic dermatits. Br J Dermatol 90:525–530, 1974
72. Elias PM, Fritsch P, Epstein EH Jr: Staphylococcal scalded skin syndrome; clinical features, pathogenesis, and recent microbiological and biochemical developments. Arch Dermatol 113:207–219, 1977
73. Lyell A: Toxic epidermal necrolysis (The scalded skin syndrome): a reappriasal. Br J Dermatol 100:69–86, 1979
74. Greer KE: Toxic epidermal necrolysis. Cutis 24:565–568, 1979
75. Baker DH, Desmond RL, Wuepper KD: The epidermolytic toxin of *Staphylococcus aureus:* its failure to bind to cells and its detection in blister fluids of patients with bullous impetigo. J Invest Dermatol 71:274–275, 1978
76. Quinn EL, Cox F, Fischer M: The problem of associating coagulase negative staphylococci with disease. Ann NY Acad Sci 128: 428–433, 1965
77. Holt RJ: The pathologic role of coagulase negative staphylococci. Br J Dermatol 86(suppl 8):42–49, 1972
78. Mitchell RG: Classification of *Staphylococcus albus* strains isolated from the urinary tract. J Clin Pathol 21:93–97, 1968
79. Sarkany I, Taplin D, Blank H: The etiology and treatment of erythrasma. J Invest Dermatol 37:283–290, 1961
80. Somerville DA: Erythrasma in young adults. J Med Microbiol 3: 57–64, 1970
81. Taplin DJ, Zaias N: The etiology of pitted keratolysis. XIII Intl Cong Dermatol 1:593–595, 1968
82. Rogosa M, Cummins CS, Lelliott RA, Keddie RM: Actinomycetes and related organisms, in Bergey's Manual for Determinative Bacteriology. Edited by RE Buchanan, NE Gibbons. Baltimore, Williams and Wilkins, 1974, pp 599–881
83. Leyden JJ, Kligman AM: Interdigital athlete's foot. Arch Derm 114:1466–1472, 1978
84. Freeman RG, McBride ME, Knox JJ: Pathogenesis of trichomycosis axillaris. Arch Dermatol 100:90–95, 1969
85. Crissey TJ, Rebell CG, Laskas JJ: Studies on the causative organism of trichomycosis axillaris. J Invest Dermatol 19:187–198, 1952
86. Hande HR, Witebsky FG, Brown MS, Schulman CB, Anderson SE Jr, Levine AS, MacLowry JD, Chabner BA: Sepsis with a new species of *Corynebacterium*. Ann Intern Med 85:423–426, 1976
87. Riley PS, Hollis DG, Utter GB, Weaver RE, Baker CN: Characterization and identification of 95 diphtheroid (group JK) cultures isolated from clinical specimens. J Clin Microbiol 9:418–424, 1979
88. McGinley KJ, Labows JN, Zechman JM, Nordstrom KM, Webster GF, Leyden JJ: Pathogenic JK group corynebacteria and their similarity to human cutaneous lipophilic diphtheroids. J Infect Dis 152:801–806, 1985
89. Larson EL, McGinley KJ, Leyden JJ, Cooley ME, Talbot GH: Skin colonization with antibiotic resistant (JK group) and antibiotic sensitive lipophilic diphtheroids in hospitalized and normal adults. J Infect Dis 153:701–706, 1986
90. Leyden JJ, McGinley KJ, Mills OH, Kligman AM: Propionibacterium levels in patients with and without acne vulgairs. J Invest Dermatol 65:382–384, 1975
91. Cunliffe WJ, Forster RA, Greenwood ND, Hetherington C, Holland KT, Holmes RL, Khan S, Roberts CD, Williams M, Williamson B: Tetracycline and acne vulgaris: a clinical and laboratory investigation. Br Med J 10:332–335, 1973
92. Rapaport M, Puhvel SM, Reisner RM: Evaluation of topical erythromycin and oral tetracycline in acne vulgaris. Cutis 30:122–136, 1982
93. Massey A, Mowbray JF, Noble WC: Complement activation by *Corynebacterium acnes*. Br J Dermatol 98:583–584, 1978
94. Webster GF, Ledyen JJ, Norman ME, Nilsson U: Complement activation in acne vulgaris: in vitro studies with *Propionibacterium acnes* and *Propionibacterium granullosum*. Infect Immun 22:523–529, 1978
95. Webster GF, Nilsson UR, McArthur WP: Activation of the alternative pathway of complement in human serum by *Propionibacterium acnes* (*Corynebacterium parvum*) cell fractions. Inflammation 5:165–176, 1981
96. Webster GF, Leyden JJ, Tsai CC, Baehni P, McArthur WP: Polymorphonuclear leucocyte lysosomal release in response to *Propionibacterium acnes* in vitro and its enhancement by sera from inflammatory acne patients. J Invest Dermatol 74:398–401, 1980
97. Puhvel SM, Hoffman IK, Reesuor RM, Sternberg TH: Dermal hypersensitivity of patients with acne vulgaris to *Corynebacterium acnes*. J Invest Dermatol 49:154–158, 1967
98. Webster GF, Leyden JJ, Nilsson UR: Complement activation in acne vulgaris: consumption of complement in comedones. Infect Immun 26:183–186, 1979
99. Puhvel SM, Hoffman IK, Sternberg TH: Presence of complement fixing antibodies to *Corynebacterium acnes* in the sera of patients with acne vulgaris. Arch Dermatol 93:364–368, 1966
100. Kaplan K, Weinstein L: Diphtheroid infections of man. Ann Intern Med 70:919–929, 1969
101. Kimbrell OC: *Corynebacterium acnes*—a cause of meningitis. NC Med J 25:516–519, 1964
102. Graber CD, Higgins LS, Davis JS: Seldom encountered agents of bacterial meningitis. JAMA 192:956–960
103. Schlesinger JJ, Ross AL: *Propionibacterium acnes* meningitis in a previously normal adult. Arch Intern Med 137:921–923, 1977
104. Beeler BA, Crowder JG, Smith JW, White A: *Propionibacterium*

acnes: pathogen in central nervous system shunt infection. Am J Med 61:935–938, 1976

105. Everett ED, Eickhoff TC, Simon RH: Cerebrospinal fluid shunt infections with anaerobic diphtheroids (*Propionibacterium spp.*). J Neurosurg 44:580–584, 1976
106. French RS, Ziter FA, Sruance SL, Smith CB. Chronic meningitis caused by *Propionibacterium acnes*. Neurology 24:624–628, 1974
107. Skinner PR, Taylor AJ, Coakham H: Propionibacteria as a cause of shunt and postneurosurgical infections. Clin Pathol 31: 1085–1090, 1978
108. Leyden JJ, McGinley KJ, Kligman AM: Role of micro-organisms in dandruff. Arch Dermatol 112:333–338, 1978
109. McGinley KJ, Lautis L, Marples RR: Microbiology of tinea versicolor. Arch Dermatol 102:168–171, 1970
110. Nazzaro-Porro M, Passi S: Identification of tyrosinase inhibitors in cultures of *Pitryrosporum*. J Invest Dermatol 71:205–208, 1978